NEW DEVELOPMENTS RELATING TO MICROBIOLOGICAL SAFETY OF APPLES

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INTRODUCTION

Outbreaks of food-borne illness have been associated with the presence *of E. coli* O157:H7, *Salmonella* and *Cryptosporidium* in unpasteurized fresh apple juice (1-3). While such outbreaks have generally been isolated and infrequent, and no illnesses have been associated with fresh apples, research on the microbiological safety of apples has been carried out to ensure avoidance of unforeseen hazards and consequent risks to safety of apple products. Previously, we reported that the efficacy of washing as a means of reducing the bacterial load on fresh apples was limited by bacterial adhesion to apple surfaces, especially at inaccessible sites, and by internalization of contaminating bacteria as might result from infiltration during processing (4). Nevertheless, laboratory trials indicated that washing with dilute hydrogen peroxide was superior to other anti-microbial treatment in inactivating *Escherichia coli* on inoculated apples (5). Recent apple washing trials, carried out with commercial processing equipment, and further investigations of the sites of bacterial contamination and survival on washed apples have suggested new approaches to the problem of apple decontamination. Other studies of interactions between plant pathogens and pathogenic *E. coli* O157:H7 on apples have revealed a potential scenario for *E. coli* proliferation on decayed apples.

APPLE WASHING TRIALS WITH COMMERCIAL EQUIPMENT

Washing trials were carried out with Golden Delicious apples, inoculated with a non-pathogenic *E. coli*, at a commercial cider mill (apple juice processor) located in Placerville, California, that was operated by the U.S. Food & Drug Administration as a research facility. Apples were washed with a flat-bed brush washer using water, commercial washing and sanitizing agents (200 ppm Cl_2, 8% trisodium phosphate, 1% acidic detergent), and 5% hydrogen peroxide, applied at 50C. None of the washing treatments were effective in reducing the bacteria population on the inoculated apples (Table 1). The apparent reduction in the cider (juice) only reflected dilution by juice made from uninoculated apples. The inability of these treatments to achieve even a 1 log (90%) reduction with the commercial brush washer was attributed to the brief exposure time of apples to the anti-microbial solutions and the inability of brushes to reach the areas on inoculated apples where bacteria were concentrated. Redesign of commercial brush washing equipment might overcome these deficiencies.

DISTRIBUTION OF BACTERIA ON APPLES

Previously, we had obtained data suggesting that bacterial populations on apples, artificially inoculated by immersion in a bacterial cell suspension, were concentrated in the area of the stem and calyx cavities at either end of the core. More definitive studies in which these portions of the apple were dissected and examined separately proved that *E. coli* were present in greater numbers (per cm^2 of surface) in these areas than on the unbroken skin surface (Table 2). Furthermore, survival of *E. coli* after washing with 5% hydrogen peroxide was substantially greater in these inaccessible sites than on the unbroken skin. Similar results were obtained in trials with apples inoculated with *Salmonella chester.* One might question whether these findings might be an artifact of artificial inoculation by immersion. However, an examination of the distribution of natural microflora on immature apples has

confirmed the occurrence of high population densities in the calyx area and a lower population density in the stem area (Table 3). Population densities were not affected by the orientation of apples on the tree (calyx facing up or down) at the time of harvest. These results suggest that development of improved methods of reducing the bacterial load on apples should focus on the sites where bacteria may be concentrated.

DECONTAMINATION OF APPLE CALYX AND STEM AREAS

A conical abrasive tool, attached to an electric drill, was used in conjunction with a 5% hydrogen peroxide/surfactant pre-wash and final rinse to remove and inactivate non-pathogenic *E. coli* in the stem and calyx areas of artificially inoculated Golden Delicious apples. This procedure was able to achieve an overall 5-log reduction in the bacterial population on the inoculated apples when the final rinse was with 5% hydrogen peroxide rather than water (Table 4). However, because the abrasive tool penetrated the skin surface and calyx interior (damage that would not interfere with juice production), this treatment could not be used for apples intended for fresh market. Therefore, we have investigated the use of a rotating brush, designed for dental hygiene, as a means of cleaning the stem and calyx areas without damage to the fruit. Promising results have been obtained when this tool was used in combination with calcinated oyster shell calcium as an anti-microbial abrasive.

INTERACTIONS BETWEEN SPOILAGE FUNGI AND *E. coli* O157:H7

Because outbreaks of foodborne illness have been associated with consumption of unpasteurized apple juice made from fruit that had fallen on the ground (3), we speculated that concomitant growth of decay organisms and *E. coli* O157:H7 might permit extensive growth of the latter organism on the normally inhospitable apple surface. Artificially wounded Golden Delicious apples were inoculated simultaneously with *E. coli* O157:H7 and one of two species of fungus associated with apple decay, *Penicillium expansum* or *Glomerella cingulata* (Fig. 1). Initially, the *E. coli* O157:H7 population increased by more than 3 logs. In the presence of *Penicillium* expansum, the surface pH decreased from 4.0 to 3.5, and the *E. coli* O157:H7 population gradually decreased. However, in the presence of *Glomerella cingulata*, the pH increased to 6.7 and the *E. coli* O157:H7 population remained constant at a level of approximately 6.3 logs. Thus, the use of decayed apples that also contained *E. coli* O157:H7 for production of unpasteurized juice potentially could result in extensive product contamination with the human pathogen. The probability of this scenario occurring should be examined by quantitative risk assessment, taking into account the prevalence of *E. coli* O157:H7 in the orchard environment, the frequency of *Glomerella* infection, the likelihood of wounding in the "dropped" apples, and ambient conditions favorable for microbial growth.

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TABLE 1. Decontamination of inoculated apples in a flat bed brush washer

	E. coli (log_{10}CFU/g)[a]			
Treatment	Before Dump Tank	After Dump Tank	After Brush Washer	In Cider (Log_{10} CFU/mL)
Water	5.49±0.09	4.92±0.37	4.81±0.26	3.83±0.15
200 ppm Cl_2	5.87±0.07	5.45±0.05	5.64±0.23	4.30±0.10
8% Na_3PO_4	5.49±0.09	5.02±0.43	4.98±0.02	3.56±0.15
1% acidic deterg.	5.87±0.07	5.49±0.03	5.42±0.50	4.28±0.05
5% H_2O_2, 50°C	5.87±0.07	5.54±0.31	5.49±0.10	4.30±0.60

[a]Mean of 4 determinations ± SD.

TABLE 2. Distribution of *E. coli* (ATCC 25922) on the surface of inoculated apples before and after washing with 5% H_2O_2 at 50°C

	$Log_{10}(CFU/cm^2)$[a]	
Location	Before Washing	Washed[b]
Skin on wedges	5.14±0.03	1.88±0.88
Skin at calyx end of core	7.26±0.01	5.48±0.51
Skin on stem end of core	6.58±0.18	5.18±0.86

[a]Based on calculated surface area of skin; determined 24 h after inoculation.
[b]Washed 1 min in 5% H_2O_2 at 50°C.

TABLE 3—Distribution of bacteria in calyx and stem areas of naturally contaminated immature apples

		Log_{10}CFU/g[a]	
Cultivar	Fruit orientation	Calyx	Stem
Rome	Calyx up	4.8±0.3	1.1±0.5
Golden Delicious	Calyx up	3.5±1.0	1.3±1.4
	Calyx down	4.8±0.7	1.8±1.3

[a]Plated on TSA.

TABLE 4. Reduction in *E. coli* (ATCC 25922) population in inoculated Golden Delicious apples by washing with 5% H_2O_2 + surfactant at 50°C and abrasion treatment of stem and calyx areas

Treatment	Log_{10}CFU/g[a]	Log_{10}CFU/g Reduction
Inoculated control	5.62	--
5% H_2O_2 + surfactant at 50°C	3.48	2.14
5% H_2O_2 + surfactant at 50°C abrasion + H_2O rinse	1.35	4.27
Inoculated control	5.73	--
5% H_2O_2 + surfactant at 50°C + abrasion + H_2O rinse	1.60	4.13
5% H_2O_2 + surfactant at 50°C + abrasion + 5% H_2O_2 rinse	0.45	5.28

[a]Mean of duplicate determinations.

Figure 1. Survival of *E. coli* O157:H7 in the presence of *Penicillium expansum* (Pen) and *Glomerella cingulata* (Glom) in puncture wounds on apples stored at room temp.